Amendments to the Specification

Please replace the paragraph beginning at page 3, line 20, as originally filed with the following amended paragraph:

Figure 1 is an alignment of the deduced amino acid sequences for human Akt-1 **(SEQ ID NO: 15)**, Akt-2 **(SEQ ID NO: 16)** and Akt-3 **(SEQ ID NO: 3)**. The sequences were aligned using the ClustalW alignment program (EMBL, Heidelberg, Germany). Amino acid residues conserved between all three proteins are included in the black areas. Residues conserved between only two of the sequences are shaded in grey. Amino acid residues are numbered in the right hand column. The conserved Thr and Ser residues that are presumed to be phosphorylated upon activation are marked with an asterisk above the sequence.

Please replace the paragraph beginning at page 19, line 16, as originally filed with the following amended paragraph:

Molecular cloning of human Akt-3.

Using the rat RAC-Pkγ sequence (Konishi et al, 1995; GenBank acc. No. D49836) as a query sequence, a BLAST (Basic Local Alignment Search Tool; Altschul et al., 1990) search was carried out in the WashU Merck expressed sequence tag (EST) database (Lennon et al., 1996) and in the proprietary LifeSeq™ human EST database (Incyte Pharmaceuticals Inc, Palo Alto, CA, USA). Several human EST clones with high similarity to the rat RAC-Pkγ were identified. One EST sequence (Incyte accession number 2573448) derived from a hippocampal cDNA library, contained part of the coding sequence including the putative methionine start codon (ATG) and part of the 5' untranslated region. The start codon was surrounded by a Kozak consensus sequence for translation start and an in-frame stop codon was present at positions –6 to –3. Based on this 239 bp sequence, oligonucleotide sense primers were synthesised for 3' rapid amplification of cDNA ends (3' RACE) experiments: Akt-3spl = 5'-ACC ATT TCT CCA AGT TGG GGG CTC AG-3' (SEQ ID No: 4) and Akt-3sp2 = 5'GGG AGT CAT CAT GAG CGA TGT TAC C-3' (SEQ ID No: 5). 3'RACE experiments were performed on human fetal brain or human cerebellum Marathon-Ready™ cDNA (Clontech Laboratories, Palo Alto, CA,

USA) according to ~~manufacturer=s~~ manufacturers ~~manufacturer=s~~ instructions using Akt-3sp1/race-ap1 as primers in the primary PCR and Akt-3sp2/race-ap2 in the nested PCR. Resulting PCR fragments were cloned and sequenced. This extended the Akt-3 coding sequence by 916 bp, but the novel sequence did not include an in-frame stop codon. A second round of 3' RACE amplification was performed on human brain Marathon-Ready™ cDNA using sense primers based on the sequence obtained in the first round (Akt-3sp3 = 5'CAC TCC AGA ATA TCT GGC ACC AGA GG-3' (SEQ ID No: 6) and Akt-3sp4 = 5' CTA TGG CCG AGC AGT AGA CTG GTG G-3' (SEQ ID No. 7)) in combination with race-ap1 and race-ap2, respectively. The sequence obtained included an in-frame stop codon and the 3' untranslated sequence up to the poly(A) tail. Antisense primers were designed based on the 3' untranslated region (Akt-3ap4 = 5'-TGC CCC TGC TAT GTG TAA GAG CTA GG-3' (SEQ ID No: 8)) and Akt-3ap5 = 5' AAG AGC TAG GAC TGG TGA TGT CCA GG-3' (SEQ ID No: 9)) and the complete Akt-3 coding sequence was amplified from human hippocampal cDNA using Akt-3sp1/Akt-3ap4 (primary PCR) and Akt-3sp2/Akt-3ap5 (nested PCR) as primers. The resulting 1200 bp PCR fragment was then cloned in the TA-cloning vector pCR2.1 (original TA cloning kit, Invitrogen BV, Leek, The Netherlands) and the inserts of several clones were completely sequenced. One clone containing an insert with the confirmed sequence (hAkt-3/pCR2.1) was used for subsequent subcloning to the mammalian expression vector pcDNA-3 (Invitrogen), yielding construct hAkt-3/pcDNA-3. In order to make a construct coding for a COOH-terminal tagged Akt-3 protein, a fragment of 553 bp was amplified from plasmid Akt-3/pcDNA-3 using an antisense primer incorporating a XhoI restriction site and the sequence coding for a hemagglutinin (HA) tag (YPYDVPDYA) (SEQ ID NO: 13) after amino acid 479 of the Akt-3 sequence. This fragment was recloned into plasmid hAkt-3/pcDNA-3 using BstEII and XhoI restriction sites yielding construct HA-hAkt-3/pcDNA-3.

Please replace the paragraph beginning at page 25, line 3, as originally filed with the following amended paragraph:

Reverse transcription (RT)-PCR analysis

Oligonucleotide primers were designed for the specific PCR amplification of a fragment from Akt-3. These primers were Akt-3sp2 = 5' –GGG AGT CAT CAT GAG CGA TGT TAC C-3' (SEQ ID No: 5) (sense primer) and Akt-3ap1 = 5' – GGG TTG TAG AGG CAT CCA TCT

CTT CC – 3' (SEQ ID No: 11) (antisense primer), yielding a 425 bp product. PCR amplifications for human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were performed on the same cDNA samples as positive controls using G3PDH primers 5' – TGA AGG TCG GAG TCA ACG GAT TTG GT-3' (sense primer) (SEQ ID NO: 10) and 5' –CAT GTG GGC CAT GAG GTC CAC CAC-3' (antisense primer) (SEQ ID NO: 14), yielding a 1000 bp fragment. These primers were used for PCR amplifications on Multiple Tissue cDNA panels (Clontech Laboratories) and on cDNA prepared from tumor cell lines. For the preparation of tumor cell cDNA, cells were homogenised and total RNA prepared using the RNeasy Mini kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. 1 Fg of total RNA was reverse transcribed using oligo(dT)$_{15}$ as a primer and 50 U of Expand^{TM} Reverse Transcriptase (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions. PCR reactions with Akt-3-specific or G3PDH-specific primers were then performed on 1 Fl of cDNA. Images of the ethidium bromide stained gels were obtained using the Eagle Eye II Video system (Stratagene, La Jolla, CA, USA) and PCR bands analysed using the EagleSight software.

Please replace the paragraph beginning at page 27, line 20, as originally filed with the following amended paragraph:

The predicted Akt-3 (Figure1) protein shows significant similarity with Akt-1 (Jones et al, 1991; 83.6% identity; 87.8% similarity) and with Akt-2 (Cheng et al., 1992; 78% identity; 84.3% similarity).The COOH-terminal `tail= has been observed in both human and rat Akt-1 and Akt-2 proteins, but it is apparently truncated in the only other reported Akt-3 sequence (rat Akt-3, Konishi et al., 1995; accession number D49836). 3'RACE experiments performed on human cDNAs derived from different tissues did not yield evidence for the existence of a shorter form of Akt-3 that would be analogous to the rat Akt-3 (data not shown). The tail in human Akt-3 comprises 28 amino acid residues (YDEDGMDCMDNERRPHFPQFSYSASGRE) **(SEQ ID NO: 12)** that replace 3 amino acid residues in the rat sequence (CPL). The tail in human Akt-3 contains a serine residue at position 472 (shown in bold) that

corresponds to Ser^{473} in Akt-1 or Ser^{474} in Akt-2. Phosphorylation of Ser^{473} and Ser^{474} has previously been implicated in the activation of Akt-1 and Akt-2, respectively (Alessi et al., 1996; Meier et al.,1997). Thr^{308} (in the kinase domain) has also been implicated in the activation of Akt-1 and this residue is also conserved in human Akt-3 (Thr^{305}).

Please replace the paragraph beginning at page 32, line 8, as originally filed with the following amended paragraph:

The sequence which has been identified represents the human homologue of Akt-3. This assignment is based on the >99% identity between the rat and human Akt-3 protein sequences. With the exception of the COOH-terminal tail seen in human Akt-3, there are only 2 amino acid differences (Gly^{10} and Ala^{396} in human Akt-3) between the rat and human Akt-3 proteins. Alignment of all the previously described Akt sequences demonstrates that Gly^{10} and Ala^{396} in the human protein correspond to Gly and Ala residues respectively in the Akt-1 and Akt-2 sequences identified from other species. Further evidence that we have identified the Akt-3 isoform comes from the presence of isotype-specific sequences represented by human Akt-3 residues 47-49 (LPY), 118-122 **of SEQ ID NO: 3** (NCSPT) and 139-141 (HHK). For each isotype, these sequences are conserved between species, but differ between the isotypes.

Please replace pages 45-51 as filed with the Sequence Listing attached hereto.